

February 8, 1993

THE GENETICS OF CORONARY ARTERY DISEASE

Peter Kwiterovich, Jr, MD*

DR. NORMA DAVIS: Our next speaker is Dr. Peter Kwiterovich, who is currently Director of the Specialized Center for Research in Arteriosclerosis at Johns Hopkins University. He graduated from Holy Cross College and then went on to Johns Hopkins where he did his MD degree. From there he did a post-doctoral fellowship in human genetics and then went on to do his internship at the Childrens Hospital Medical Center in Boston. He came back to Johns Hopkins where he did his residency in pediatrics. He also serves as the Chief of Lipid Research, Arteriosclerosis Division in the Dept. of Pediatrics at Johns Hopkins and he's going to talk to us about "The Genetics of Coronary Artery Disease," which is probably something that's of great interest to all of us. (applause)

DR. KWITEROVICH: Well, thank you very much. It's an appropriate topic just before lunch. Coronary heart disease is the major health problem in the United States, causing over a half million deaths each year, one each minute, and 680,000 hospitalizations for myocardial infarction. There are over 5 million people with diagnosed coronary disease, and many more Americans, perhaps as many as 20 million, have asymptomatic coronary disease. This has a direct health cost of \$8 billion dollars a year, and a total economic cost of \$60 billion dollars a year.

Coronary heart disease results from a process called atherosclerosis, which begins around the age of 10 years, as early lesions called fatty streaks, a deposit of cholesterol ester in the lining of the coronary artery. Throughout the next several decades some of these fatty streaks progress into fibrous plaque lesions characterized by a cap overlying a nidus of cholesterol ester-rich material. Some of these fibrous plaque lesions may undergo calcification, hemorrhage, ulceration and thrombosis, leading to a more advanced lesion, which can lead to myocardial infarction or stroke, peripheral vascular disease, etc.

Individuals who may have inherited genetic predisposition to either dyslipidemia, to hypertension or diabetes, for example, will develop earlier lesions of atherosclerosis, so-called premature coronary artery disease and premature hardening of the arteries.

Now the traditional risk factors for coronary artery disease are hyperlipidemia, low levels of HDL cholesterol, obesity, hypertension, cigarette smoking and diabetes, which explain about 50% of the risk for developing coronary artery disease. In regard to genetic information, I will be focusing on the dyslipidemias, low HDL, and somewhat on hypertension. I will also touch briefly on diabetes.

I'll be talking about some of the research that we and others have been doing to explain the 50% of coronary artery disease that is not currently well understood, with the focus on the apolipoproteins.

The lipoproteins are present in blood. There are over a dozen apolipoproteins, and they possess characteristics that enable them to interact with lipid. They have a non-polar surface, and then the other side of the peptide has a polar surface which enable them to interact with the aqueous environment of plasma. So they permit lipid particles to be transported, but they also have functions as co-factors and to interact with certain receptors on the surface of cells.

In addition to triglyceride-rich lipoproteins (chylomicrons and VLDL), we have the cholesterol-rich lipoproteins, the low density lipoproteins or LDL, and apolipoprotein-B, which is a major apolipoprotein of LDL and also on the triglyceride-rich lipoproteins, HDL, high density lipoproteins, and apo A-I, the major apolipoprotein of HDL. The apolipoproteins are named according to alphabetical nomenclature, A,B,C,D,E,F,G, etc., but we're primarily talking about A-I, B, C's and E's.

Now I'd like to talk about the genetic disorders in the various lipoprotein transport systems and what's known about them. Dietary fat and cholesterol comes into the small intestine, is mobilized by the lymphatics. Chylomicrons, which have apo E, apo B-48, which enables it to be mobilized out of the intestine, and apo C's, particularly apo C-II. The chylomicrons are then in the surface of the capillary. The triglycerides are hydrolyzed by lipoprotein lipase, through the assistance of apo C-II which is a co-factor. You then have the production of remnant particles, which are taken up to the interaction of apo E in the chylomicron remnant receptor. There are some genetic defects in both lipoprotein lipase, but also in apo C-II, producing chylomicronemia syndrome.

* This article is being printed without author review in order to meet printing schedules.

In lipoprotein lipase deficiency the enzyme either is not present or is not made in a functional state. In point of fact, only 1 in a million people have this mutation in lipoprotein lipase. However, 1 in 500 individuals are carriers for the defect in lipoprotein lipase, and as we shall see, there's now evidence that the carriers for the defect for lipoprotein lipase may, in fact, have premature coronary artery disease. The chylomicron will not be hydrolyzed by lipoprotein lipase in the absence of the co-factor, apo C-II.

The clinical findings in the chylomicronemia syndrome are: recurrent, acute pancreatitis, eruptive xanthomas, retinalis, hepatosplenomegaly, autosomal recessive inheritance. You will see that premature coronary artery disease is not a component of a chylomicronemia syndrome, because the chylomicrons are too large to enter into the vascular wall. However, the acute pancreatitis can be a life-threatening circumstance. And I expect that gene therapy will be proposed for children who have lipoprotein lipase deficiency within the next five years. Usually the mutations of current exons 3, 4 and 5 are on the lipoprotein lipase gene. So, it's a fairly rare genetic trait, which, in the homozygous state, is associated with a life-threatening circumstance, but not premature coronary disease. But in the heterozygous carriers, it is probably associated with premature coronary disease. I'll come back to that later, when I talk about familial combined hyperlipidemia.

Now I'd like talk about some genetic defects in the genous VLDL pathway. The VLDL molecules, which are rich in triglyceride, have apo C-II on the surface, apo B-100, which is the major protein of VLDL and LDL, and apo E. The VLDL is hydrolyzed by lipoprotein lipase produced in free fatty acids, producing a VLDL remnant. Some of these remnants may be taken up through the interaction of apo E, with the LDL receptor on the surface of liver cells. Whereas, others may be acted upon by hepatic lipase and converted into the cholesterol-rich LDL, which is then removed by the LDL receptor on the surface of liver cells.

One of the most common causes of premature coronary disease is an overproduction of apolipoprotein B in the liver, leading to an overproduction of VLDL, leading to a flooding of this cascade, giving you an increased number of apo B-containing lipoproteins, which are atherogenic.

I wish that we could really say we knew what the biochemical basis for this defect is, but we don't. We've been very interested in studying the apolipoprotein B-100. This is very common in coronary artery disease and accounts for about 10% of all families with prema-

ture coronary artery disease. Cholesterol and/or triglyceride levels are elevated, usually only to a moderate extent, reflecting increased LDL and/or VLDL. HDL may be low, characterized by an overproduction of apo B-100, and coronary heart disease risk is increased.

The monogenic hypothesis for familial combined hyperlipidemia was that the basic problem resided in the liver, with overproduction of apolipoprotein B-100 leading to an increased production of VLDL particles, which then led to an increased number of VLDL remnants and increased number of LDL, all promoting premature atherosclerosis. So, a logical candidate would be apolipoprotein B gene, a regulatory portion of the apo B gene. The apo B gene is on human chromosome 2. We took advantage of some of the lipid studies that John Phillips talked about, and using haplotypes for the apo B gene and PCR, we looked at the linkage between apo B gene and apo B levels in a number of families with premature coronary artery disease, and published the data in the *American Journal of Human Genetics* in 1992. We found a χ^2 score of -7, and one other group has confirmed this, so we feel that the apolipoprotein B gene is not the major gene involved in familial combined hyperlipidemia.

That leaves one to consider other genes, perhaps the presence of several other genes. There is now evidence from the Seattle group that lipoprotein lipase is deficient in families with familial combined hyperlipidemia. And in point of fact, John Bonzel has stated that one-third of families with familial combined hyperlipidemia have a 50% decrease in the mass of lipoprotein lipase. His laboratory and others, including ours, are now looking at the lipoprotein lipase gene in these families to see the extent of the abnormalities that may be present, particularly in exons 3,4 and 5 in such families.

Some have proposed that a defective apo B-100, might reflect familial combined hyperlipidemia. There is a known mutation in apo B-100 itself, residue 3500, in which a positively charged arginine is replaced with a neutral amino acid. Such patients produce VLDL and then the remnant, and they have an increased number of LDL particles, not because the LDL receptor is faulty but because the ligand itself is deficient. Using PCR, we looked at our family collection of premature coronary disease, and we did not find one case of apo B defective 100 in premature coronary artery disease. It may be more prevalent later in life, for garden variety coronary artery disease, but it certainly is not prevalent in premature coronary artery disease, although it occurs in the general population at a frequency of 1 in 500.

There's another condition called familial hypertriglyceridemia, that often occurs in families. It's rare in its severe form. Its primary genetic basis is unknown, which usually is characterized by elevated VLDL. Now there's a lot of interest in the fact that VLDL can be atherogenic, as well as LDL. Total cholesterol is only moderately elevated, LDL cholesterol is actually lower and HDL cholesterol can be reduced.

In familial hypertriglyceridemia, unlike familial combined, you have a true phenotype. In other words, a high triglyceride is passed on consistently within the family. In familial hypertriglyceridemia, there's not an overproduction of apo B-100, but an overproduction of triglycerides in the liver, such that the particles that come out are very triglyceride enriched. So although the lipoprotein lipase enzyme is normal it can't interact with the large triglyceride-rich VLDL particles, which pile up in the blood. We don't know the biochemical basis for this syndrome at the present time, but I'll show you data on its prevalence in premature coronary artery disease a little bit later.

Now I'd like to spend a few minutes on familial hypercholesterolemia (FH), which is probably well known to most of you, and which may be making an impact on the insurance industry soon, because of the possibility of gene therapy. FH heterozygotes occur in about 1 in 500 in a general population. If two of them marry, there's a 1 in 4 chance that the disease will be passed on to a child. And the rate of the FH homozygote is 1 in a million, since there's a 1 in 250,000 chance that heterozygotes would marry.

The phenotype is expressed early in life at birth. We see FH heterozygous children with an average cholesterol of 300 and LDL close to 250. The FH homozygous children have a cholesterol close to 700 and LDL over 600, with the lowest HDL cholesterol. Adults who carry the mutant allele usually have a cholesterol of about 350, LDL close to 300, with normal triglyceride levels.

The adults who are FH heterozygous often have tendon lymphomas. In regard to coronary disease, the estimated risk in percent of FH heterozygotes developing coronary disease is 20% by the age of 40 years, almost 50% by 50 years of age, with 1 out of 4 being dead, and by 60 years of age, 75% will have coronary disease. The female heterozygotes lag behind the males by 10 years, so that by 50 years, 20% have coronary disease and 60 years, half of the women with this trait have coronary disease.

If the two mutant alleles come together in the same person, these children will develop coronary disease

very early. There's a lot of interest now, particularly from the Michigan group, in giving the LDL receptor gene, which I'll talk about in a moment, to these FH homozygous children. This would be very cost efficient in the long run, because except for plasma exchange therapy, the only other therapy now available would be a liver transplantation, which we and the NIH group no longer recommend routinely for such children.

The LDL receptor gene, many of you may be familiar with it. It does have a ligand binding domain where positively charged residues of apo B, such as arginine and lysine, interact with negatively charged residues of the ligand binding. There is a middle section which is homologous to the epidermal growth factor. Mutations can occur anywhere along the LDL receptor gene.

The LDL receptor pathway involves the uptake of the cholesterol-rich LDL by the cell surface receptors, by endocytosis. It's broken down into free cholesterol, which can decrease further cholesterol synthesis and further production of LDL receptors.

There are now over 100 mutations in the LDL receptor gene. There's a French Canadian mutation published in the *New England Journal of Medicine*, which prevents the formation of messenger RNA in a single sequence. Exons 2,3,4 and 5 can affect ligand binding with decreased binding in transport.

If one wanted to screen in the general population using DNA methods, it would be not very efficient. If you use the PCR probe, for example, it would pick up a particular mutation and then you would miss all the others. There are so many mutations that it's impossible to screen for just one in general population. On the other hand, if you identify a mutation in a subgroup, you could then use PCR methods to screen the population. In a family you could use a recombinant DNA method. but for general population screening, it would not be suitable.

Now the hypothesis is that modified LDL, either through oxidation which can affect the unsaturated areas of the molecule or can also affect apo B polypeptide itself, or by glycosylation which occurs in diabetes, makes the LDL go through the receptor independent pathway. Entering into the cell is unregulated, so the LDL keeps piling in and you get a deposition of cholesterol ester.

We now know in humans that if we feed mono-unsaturated fats such as is found in olive oil, that we change the composition of the core of the LDL and it's less susceptible to oxidation because mono-unsaturated fats

have one unsaturated bond, compared to polyunsaturated fats. The anti-oxidants, beta carotene and vitamin E, are carried in the core of the LDL molecule, and if you feed humans beta carotene or vitamin E and isolate their LDL, it's much less susceptible to oxidation in vitro. Vitamin C is also an anti-oxidant which appears to prevent the oxidation of LDL and also the adverse effects of cigarette smoking on the oxidation of LDL.

So this is, I think, very simple preventive measures that we can hopefully institute in this country in regard to nutrition to get around abnormalities in LDL metabolism.

I wanted to briefly comment on another disorder called remnant hyperlipidemia, again, to show how humble we really must be in terms of trying to understand the genetics of premature coronary disease. Patients with remnant hyperlipidemia, like those with familial hypercholesterolemia, can have xanthomas, xanthelasmas, corneal arcus, premature coronary artery disease and also peripheral vascular disease and cerebral vascular disease, if associated, gout and diabetes.

We know that there is a polymorphism for apolipoprotein E. It occurs in the general population and someone who is an E-II, E-II, both their genes are giving a apo E molecule in which residue 112 and 158 consist of _____ rather than arginine. E-III, there's _____ at 112 and arginine at 158, and E-IV, E-IV, arginine at both positions. Now patients with remnant hyperlipidemia are invariably E-II, E-II, is about one percent incidence in the general population. The total LDL cholesterol is actually lower, VLDL is increased. But only 1 out of 150 homozygous people for apo E-II develop a full-blown type III.

With E-III there's a normal uptake through the remnant receptor. And with VLDL remnants or IDL for E-III, there's a normal uptake through the LDL receptor or normal conversion into LDL. If you carry the E-II alleles, you don't remove dietary fat as efficiently, and you don't take up VLDL as efficiently. But yet one still needs another gene to explain the fact that only 1 out of 50 people with E-II develop the full-blown type III, and this gene relates to apo B overproduction.

So in this particular case, it seems to be a double hit. So even if we could screen for everybody for apo E-II, and if we just wanted to screen for those at those two particular places in the gene, we still will not be able to identify the ones most at risk for developing coronary artery disease. So we still have a long way to go to identify even people with genetic defects with premature coronary disease.

Now I'd like to comment on HDL metabolism. Many of you heard about HDL, so called "good cholesterol." It's round and it's flat and made in the intestine and the liver and has apo A-I as its major protein, and then probably through the interaction of a HDL receptor on peripheral cells, it removes free cholesterol and through cholesterol ester transferase, makes a sphere, HDL-III, which looks like a baseball, with a cholesterol ester in the middle. HDL actually goes back into the liver, directly to the liver. This is the reverse cholesterol transport, and people who have good reverse cholesterol transport get much less coronary artery disease. The cholesterol and HDL can be transferred up to other lipoproteins, which can then take the cholesterol into the liver through the LDL receptor pathway. Also, the chylomicrons and VLDL, the triglyceride-rich molecules, transfer surface material up to HDL, making it a more mature particle.

Many patients with hypertriglyceridemia will have low HDL, not because of a defect in apo A-I gene but secondary to hypertriglyceridemia. This is the frustrating thing about genetic studies of low HDL levels, which might help us pick out people at risk for coronary disease. One can have an apo A-I deficiency, a deletion in the A-I, C-III, A-IV gene complex, with markedly low levels of HDL and a very high susceptibility to coronary disease. On the other hand, you can have a single point mutation like apo A-I, and the HDL levels are low, but they don't all get coronary disease susceptibility. Then you have all the other genes where the HDL levels are very low, and they may or may not have susceptibility for coronary disease. So it's very difficult to go from the epidemiologic observations of a low HDL to try and figure out what's going on on a genetic basis.

If one looks at the prevalence of these various defects in survivor's of premature myocardial infarction, you see that only about 20% fit these monogenic syndromes. As a matter of fact, you've seen that familial hypercholesterolemia, the one that's best elucidated the LDL receptor defect, only explains 4%. That really means that for 95% of premature coronary disease, we don't understand the fundamental genetic basis of it very well.

We've been interested in the genetics of premature coronary disease and through support from NIH have established a study population of about 100 men and 100 women undergoing electrodiagnostic coronary arteriography for premature coronary atherosclerosis. I don't have time to talk about all the results of the study, but suffice to say that we have collected a large number of families in which we're studying the genetics of premature disease. One of the things that we described

in collaboration with Alan Steinman about 10 years ago is what we call the hyperapo B phenotype. This is the absence of type III, LDL cholesterol of less than 90th percentile. It's not a type II-A or type II-B. The LDL B is elevated greater than 130. The triglyceride can be elevated above the 90th percentile, or normal. Now when I analyzed the data from the index cases from the Johns Hopkins study, and this will be reported next month in the *American Journal of Cardiology*, I found that 34 percent of people with premature coronary disease had this hyperapo B syndrome, with either normal triglycerides or high triglycerides. Type II-A was 11.9 percent, and I was surprised that type IV was 16.5 percent. Hypo alpha, after all these other things are considered, 3.7 percent. Isolated high LPA, 73.% which I'll come back to in a moment and only 20 percent when normal. So we found that hyperapo B is not linked to the apolipoprotein B gene itself. Several years ago I published a paper in which we described normal human serum basic proteins. Basic protein 1, 2 and 3 have molecular weights of 14,000, 27,500 and 55,000. In normal cells, they will stimulate the incorporation of fatty acid in the triglyceride. And in abnormal cells, from patients with hyperapo B, are abnormal effects.

I'm just going to summarize. These new serum basic proteins are going to provide new markers, particularly with their receptors, in understanding the genetics of premature coronary disease. Basically, there are two defects in hyperapo B: delayed clearance of dietary fat from the intestine, and overproduction of apo B in VLDL in the liver, leading to an increased number of these small dense LDL particles, characteristic of hyper apo B.

One defect appears to be an adipose tissue where basic protein 1, also called acetylation stimulatory protein by Sneiderman, stimulates normal incorporation of free fatty acid in the triglyceride. A defect in this process, in hyperapo B leads to a delayed clearance of post-prandial dietary fat. We originally thought that there might be an increased flux of free fatty acids in the liver, driving triglyceride production and VLDL production. However, my work in cultured fibroblasts with patients with hyperapo B has shown a six-fold increase in the mass of cholesterol ester, with basic protein II. There is now evidence that cholesterol esters are the culprit that drive apo B production. So, we think that there are a sizable number of people with premature coronary artery disease who have a defect in this putative receptor for the basic proteins. So I think that you'll be hearing more about that in the next 3-5 years.

I did want to mention that the basic proteins also appear to be involved in carbohydrate metabolism and this

leads me to comment about diabetes and the work of Ravin, what he calls Syndrome X. This is a resistance to insulin-stimulated glucose uptake, hyperinsulinemia, hypertension, glucose intolerance, increased VLDL, decreased HDL, and these people have small dense LDL. My own thinking is that there is going to be an inter-relationship between the hyperapo B and hyperinsulinism.

In regard to diabetes, it's very frustrating, because people have looked at the insulin gene in patients with type II diabetes, and except for rare families, there are no abnormalities in the insulin gene. As a matter of fact, they've looked at the glucose transporters, and there's no systematic abnormality in the glucose transporters either. So, diabetes is also elusive, just as garden variety coronary artery disease is.

I wanted to comment briefly about a new risk factor called Lp(a). This is an apo B-100, LDL molecule hooked up through a disulfide bridge, to apo A, which is a molecule homologous to plasminogen. This is under strong genetic influence from multiple alleles. There is a wide range of plasma, 1-340. This is one time in life where zero is better. There's an increase in coronary disease risk, going from 1.5 fold to greater than fifty. This is relatively resistant to therapy, although _____ has some fascinating data. Linus says that Vitamin C makes Lp(a) go down. But none of the conventional lipid lowering drugs will do that, except for niacin. It is very prevalent in blacks and we're now examining a black population to see how important Lp(a) is for vascular disease in the black population. The concentration of Lp(a) is inversely related to the size of the polymorphic form present. So the smaller the apo A, the higher the level in the blood. And this is related to regions which are homologous to plasminogen. Some feel that this is a risk factor because it promotes thrombosis. And there's a lot of interest in this being possibly an interrelationship between thrombosis and atherosclerosis.

I conclude my presentation with a few comments about hypertension. This is not an area in which I do active investigation. So, I've reviewed the work of Roger Williams and co-workers from Utah pedigrees, and that's what I'll present to you as a model for genetics of hypertension.

They looked at predictive strength of a positive family history in 7,625 families from Utah. They found that 32% of people with hypertension had a positive family history for hypertension. But if you had a mild family history of hypertension, the relative risk in men and women from 20-39 and 40-49, etc., was increased, in

both the men and the women, 2.8, 3.2-fold, etc. If there was a strong family history of hypertension, that is two siblings before the age of 55 had a history of hypertension, this accounted for a smaller proportion, 11% of hypertension. But the relative risk in the relatives was much higher, 4.1 and 5-fold. A higher relative risk if you had a stronger family history. When Dr. Williams looked at the family history for coronary heart disease in the Utah families, a mild family history, being one sibling before age 55, found in 13% of the families, significant increase in the relative risk in the other people in the family for having coronary heart disease. If there was a strong family history of coronary disease, two siblings before age 55, found in only 2% of the group, a very high odds ratio, 12.78, 8.6, 10.4, for early coronary disease in both the men and women from these families.

Now an interesting thing that came out of these studies was that Williams found that lipid abnormalities were very common in this syndrome that he called familial dyslipidemic hypertension. He took 131 siblings with early hypertension from 58 families, and he defined this syndrome called familial dyslipidemic hypertension, or FDH, which is defined as the presence of hypertension in the index case and hypertension in one sibling before the age of 55 years. He then looked at the lipid abnormalities in these people with familial dyslipidemic hypertension, and he found that 19% had LDL above the 90th and 30% triglyceride above the 90th percentile and 39% had HDL below the 10th percentile, 65% one of the above. The ratio observed was significantly increased for lipid abnormalities. So, this is why the syndrome is called "familial." This led to the description of a subset, familial dyslipidemic hypertension or FDH, in which one had both hypertension and lipid abnormalities. I find this very interesting because it's trying to tell us something that we should be paying attention to in terms of our research.

He also looked at the prevalence of high blood pressure in the various subsets. 11% of all Utah population had hypertension. Hypertension before age 60, probably familial hypertension, was 51% of all hypertensive subjects, and 6% of the total population.

Hypertension before age 60 in two or more siblings was 25% of all hypertensives, and 3% of the population. And familial dyslipidemic hypertension, early hypertension and dyslipidemia before age 16, two or more siblings, was 12% of all high blood pressure and 1% of population.

Coronary-prone families were 2-6% of the Utah population, and comprised 50% of those with early coronary disease. Now 5,304 of the 222,546 people had coronary

artery disease before 55 years of age. This was 9% of all families. A sample of 20 families with early coronary disease and their controls showed that those with early coronary disease who also had early hypertension were 39%, 34% had cholesterol above the 90th percentile, 20% LDL, 39% triglyceride and 36% low HDL. So this again points out that in families with early coronary disease a significant proportion of them had both early hypertension and lipid abnormalities.

Williams has found that the lipid abnormalities precede the hypertension by about ten years, in his experience.

I think it's fascinating, showing this interrelationship between dyslipidemia and hypertension. People have also speculated about interrelationships of dyslipidemia and hypertension and insulin resistance. There is some evidence that dyslipidemia can be related to insulin resistance. Many people with dyslipidemia have insulin resistance. Insulin resistance itself may make one predisposed to familial dyslipidemic hypertension.

Low exercise, obesity and high fat diet are environmental variables that may affect insulin resistance. Dyslipidemia can also affect platelet reactivity and may also affect the surface of endothelial cells. So there appears to be a constellation of interaction. We talked about a lipase mutation, increased apo B turnover producing dyslipidemia, and through the work with the serum basic proteins, I think we may find an interrelationship between the increased apo B turnover and insulin resistance in dyslipidemia.

I wanted to conclude my presentation by saying that the future will hold other genetic studies about the vascular wall. There's a lot of work going on now concerning the vascular wall. Apparently, genetic factors are going to affect the endothelium which lines the blood vessel wall, and also the smooth muscle cells which are underneath. They provide vascular tone, normally they retard platelet and leukocyte adhesion. They inhibit smooth muscle cell migration and proliferation. And they're a barrier to LDL, degraded VLDL, etc. There's now evidence that LDL itself, that hypertension, diabetes and cigarette smoking, all can cause abnormalities in the endothelium, leading to endothelial dysfunction. Vasoconstriction, increased platelet and leukocyte adhesion, increased smooth muscle cell migration and growth and increased lipid deposition. So there probably will be new genes particularly related to growth factors in cellular phenotypes which will contribute further to our understanding of genetics of premature coronary disease. Thank you. (applause)